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ABSTRACT

Heat exposure impairs human blood pressure control and markedly reduces tolerance to a simulated hemorrhagic challenge. Inspiratory resistance breathing enhances blood pressure control and improves tolerance during simulated hemorrhage in normothermic individuals. However, it is unknown if similar improvements occur with this maneuver in heat stress conditions. This study tested the hypothesis that inspiratory resistance breathing improves tolerance to simulated hemorrhage in individuals with elevated internal temperatures. On 2 separate days, 8 subjects performed a simulated hemorrhage challenge (lower body negative pressure, LBNP) to pre-syncope following an increase in internal temperature of 1.3±0.1 °C. During one trial subjects breathed through an inspiratory impedance device set at 0 cmH_20 of resistance (Sham), while on a subsequent day the device was set at -7 cmH_20 of resistance (ITD). Tolerance was quantified as cumulative stress index (CSI). Subjects were more tolerant to the LBNP challenge during the ITD protocol as indicated by a >30% larger CSI (Sham: 520±306 mmHg × min; Experimental: 682±324 mmHg × min, $P<0.01$). These data indicate that inspiratory resistance breathing modestly improves tolerance to a simulated progressive hemorrhagic challenge during heat stress.

New Findings

What is the central question of this study?

Does inspiratory resistance breathing improve tolerance to simulated hemorrhage in individuals with elevated internal temperatures?

What is the main finding and its importance?

The main finding of this study is that inspiratory resistance breathing modestly improves tolerance to a simulated progressive hemorrhagic challenge during heat stress. These findings demonstrate a scenario in which exploitation of the respiratory pump can ameliorate serious conditions related to systemic hypotension.

INTRODUCTION

Military operatives deployed in hot environments, especially desert climates, are exposed to considerable heat stress (Welles et al., 2013). A combination of elevated environmental temperatures, increased metabolic heat production due to demanding physical activity, and clothing/body armor can cause internal temperatures of soldiers to rise by more than 2 °C (Buller et al., 2008). According to the U.S. Armed Forces Health Surveillance Branch, heat-related health conditions remain a significant threat to the safety and operational effectiveness of military personnel (Armed Forces Health Surveillance Bureau, 2017). One medical complication that has been well documented in hyperthermic conditions is compromised blood pressure control (C. G. Crandall & Gonzalez-Alonso, 2010; C. G. Crandall & Wilson, 2015; Horvath & Botelho, 1949; Keller et al., 2009; Lind, Leithead, & McNicol, 1968; Nunneley & Maldonado, 1983; Schlader, Wilson, & Crandall, 2016; Shvartz & Meyerstein, 1970; Wilson, Cui, Zhang, & Crandall, 2006; Yamazaki, Monji, Sogabe, & Sone, 2000). This results in an overwhelmingly earlier incidence of cerebral hypoperfusion and syncope during a hemorrhagic injury, relative to if the individual was normothermic (Bain, Nybo, & Ainslie, 2015; Wilson et al., 2006). Because hemorrhage from major trauma is the leading cause of death on the battlefield (Eastridge et al., 2011), the combination of heat stress and hemorrhage increases the lethality of a battle injury.

Prior investigations have demonstrated the effectiveness of inspiratory impedance as a resuscitative countermeasure against organ hypoperfusion during hemorrhage in normothermic conditions (Lurie et al., 2004; Sigurdsson et al., 2006; Yannopoulos, Metzger, et al., 2006; Yannopoulos, McKnite, Metzger, & Lurie, 2006).

Mechanistically, inspiratory impedance decreases intrathoracic pressure, which enhances venous return and increases cardiac output and mean arterial pressure (Convertino et al., 2004). Inspiratory impedance can be achieved with an impedance threshold device (ITD). This device is composed of a rubber valve and mechanical spring which induces negative pressure at -7 mmH_2O during inspiration (Convertino et al., 2011).

In a normothermic state, the ITD improves tolerance to a simulated hemorrhagic challenge (Kay, Sprick, & Rickards, 2017; Rickards, Ryan, Cooke, Lurie, & Convertino, 2007). In swine, the ITD is effective in the rescue of hypotension due to heat stroke (Voelckel, Yannopoulos, Zielinski, McKnite, & Lurie, 2008). It is unknown whether this device is equally effective in improving tolerance to a hemorrhagic challenge in heat stressed humans. Therefore, the purpose of this investigation was to examine the effect of inspiratory impedance on tolerance to a hemorrhagic challenge in a hyperthermic state. We hypothesized that utilizing the ITD would better preserve blood pressure and improve tolerance to simulated hemorrhage in heat stressed humans.

MATERIALS AND METHODS

Subjects and Ethical Approval

Eight subjects (7 males) that were not taking any medications, normotensive, non-smokers, and free of any known cardiovascular, metabolic, or neurological diseases volunteered to participate in this study. Descriptive characteristics (mean ± SD age) include: age, 29 ± 5 years; height, 180 ± 5 cm; and weight, 75 ± 4 kg. Subjects

were instructed to refrain from alcohol, caffeine, and exercise for 24 hours prior to the experimental trials. All subjects were informed of the purpose, procedures, and risks of the study before providing written informed consent. The protocol and consent were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas (IRB # 102007-013). This research project conformed to the Declaration of Helsinki.

Instrumentation

In order to assess internal temperature (T_{core}), subjects swallowed an ingestible telemetry pill (HQ Inc., Palmetto, FL, USA) approximately 2 hr prior to start of data collection. Also prior to data collection, euhydration was confirmed with a urine specific gravity measurement of less than 1.025 utilizing a digital refractometer (Atago, Japan). Heart rate was measured from an electrocardiogram (ECG, Agilent, Munich, Germany) that was interfaced with a cardiotachometer (1000 Hz sampling rate, CWE, Ardmore, PA, USA). Non-invasive arterial blood pressure was continuously measured utilizing photoplethysomography (Finometer Pro, FMS, Amsterdam, Netherlands). Arterial blood pressure was also obtained via auscultation of the brachial artery (SunTech Mecical Instruments, Raleigh, NC, USA). Respiratory rate and the partial pressure of end-tidal carbon dioxide ($EtCO_2$) were acquired from a nasal cannula connected to a capnography (9004 Capnocheck Plus; Smiths Medical Internaltion Ltd, Watford, Herts, UK).

Heat stress was imposed passively using a tube-lined water perfusion suit, which covered the entire skin surface area except for the feet, hands, one forearm, and the

head (Allen-Vanguard Technologies Inc., Ottawa, ON, Canada). Laser Doppler flux (LDF; an index of skin blood flow) was measured on the skin of the exposed dorsal left forearm via a laser-Doppler probe (Periflux413; Perimed, North Royalton, OH, USA) connected to a laser-Doppler flowmeter (Periflux5010; Perimed). All measurements were obtained from participants lying in the supine position on a patient bed.

Lower body negative pressure (LBNP) tolerance testing

A LBNP tolerance test is a commonly utilized experimental technique to simulate a hemorrhage challenge (Cooke, Ryan, & Convertino, 2004; Hinojosa-Laborde et al., 2014). In this model, blood is redistributed to the lower extremities by application of sub-atmospheric pressure to the lower body, effectively reducing central venous pressure and venous return (i.e. central hypovolemia) (V. A. Convertino, 2014; Cooper & Hainsworth, 2001). An incremental LBNP protocol was used to determine maximum tolerance. This incremental protocol begins with applying 20 mmHg of negative pressure for 3 min, with increases of 10 mmHg of negative pressure every 3 min until test termination. Termination of LBNP was based on at least one of the following criteria: a) continued reports by the subject of feeling faint and/or nauseous; b) a rapid decline in blood pressure resulting in systolic blood pressure less than or equal to 80 mmHg; and/or c) a relative bradycardia accompanied with narrowing of pulse pressure.

A cumulative stress index (CSI: mmHg*min) was used to quantify maximum tolerance to the progressive LBNP. This index is determined mathematically by summing the product of the negative pressure and time, in minutes (or fraction of min),

for each stage (e.g., 20 mmHg X 3 min + 30 mmHg X 3 min + 40 mmHg X 3 min, etc.) until test termination (Levine, Lane, Buckey, Friedman, & Blomqvist, 1991).

Experimental protocol

Participants volunteered for two experimental trials separated by a minimum of 4 days, with the experimental protocols for each subject initiated at the same time of day. All experimental trials were performed at an ambient room temperature of 22-23 °C. Day 1 was the control trial (SHAM) and day 2 was the ITD trial, utilizing ITD as a countermeasure. The protocol dictated the need to identify tolerance with the SHAM trial first, and then to use this tolerance information for the ITD trial. Therefore, the experimental days were not randomized. For both experimental days, following instrumentation, subjects first rested in the supine position for 30 min while 34 °C water perfused the tube-lined suits. Following normothermic baseline measurements, whole-body heating commenced by circulating 48-50 °C water in the suit until an increase in T_{core} of approximately 1.3 °C was observed. Upon reaching the desired change in T_{core}, the temperature of the water perfusing the suit slightly reduced to maintain T_{core} at the desired elevated temperature and prevent a further increase in T_{core}. The ITD face mask was then applied and the graded LBNP testing protocol initiated.

During LBNP in the SHAM trial, no inspiratory resistance (0 cmH_20) was engaged through the ITD device. For the experimental trial, the same facemask was used throughout but with the ITD device engaged (at 7 cmH_2O) at the start of the LBNP stage immediately prior to that at which pre-syncope occurred in the SHAM trial.

Data analysis

All experimental data were sampled at 1000 Hz on a 16-channel data acquisition system (Biopac, Santa Barbara, CA, USA). Analysis of the cardiovascular, respiratory, and thermoregulatory variables were determined from the final min of normothermic baseline, the final min of the whole-body heating conditions prior to LBNP, and the final 20 sec of respective LBNP stages. Mean arterial pressure (MAP) acquired from brachial auscultation was derived using an adjusted mathematical equation to account for the proportional changes of time spent in systole and diastole as a function of heart rate (Moran et al., 1995). These values were analyzed and reported for steady-state conditions, i.e. normothermic baseline and hyperthermia prior to LBNP. Finometer MAP was analyzed throughout LBNP due to the dynamic nature of the cardiovascular measurements induced by this stress. Cutaneous vasodilation was indexed as cutaneous vascular conductance (CVC = arbitrary units/MAP) and was analyzed based on relative changes during the heat stress period just prior to the onset of LBNP.

Comment [MH1]: Comment 13

Cardiovascular and respiratory responses during LBNP were compared between SHAM and ITD trials at three distinct time points. T3 indicates the point at which presyncope occurred, resulting in LBNP test termination, during the SHAM trial. That same time point (i.e., from the onset of LBNP) was identified for the ITD trial, which reflects the maximal common duration of LBNP for both trials. T2 represents the time point one completed LBNP stage prior to T3 in both SHAM and ITD trials. Notably, the ITD device was engaged at the start of this LBNP stage during the ITD trial. T1 represents two full LBNP stages prior to T3 in both trials. For the one subject that

achieved presyncope during the SHAM trial before completing two full LBNP stages, data for T1 were analyzed from the period of time just prior to the initiation of LBNP.

Statistical Analysis

All values are presented as mean ± standard deviation (SD). A paired-t test was utilized to compare the differences in CSI between the two conditions (SHAM vs ITD). A two-way repeated measures ANOVA (condition and time) was used to compare the cardiovascular and respiratory variables at T1, T2, and T3 between SHAM and ITD trials. If a significant interaction was observed, post-hoc multiple comparisons were performed utilizing paired t-tests with a Sidak adjustment. Paired t-tests were performed on baseline measurements and responses to whole-body heating of the two experimental trials. All analysis and graphing was conducted using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA USA). Statistical significance was accepted at $P<0.05$.

RESULTS

The cardiovascular and T_{core} measurements at normothermic baseline and hyperthermic conditions are presented in **Table 1**. No differences were identified for any of the variables (HR, MAP, respiratory rate, T_{core}) between trials at these two steady-state conditions. Increases of internal temperature induced by whole-body heating prior to LBNP testing were similar (1.3±0.1 °C; P=0.59) between the two experimental trials. This observation held consistent with the results of the ANOVA

analysis, demonstrating no differences in T_{core} responses at T1, T2, and T3 between conditions (interaction, P=0.73; condition, P=0.16; and time, P=0.13).

Comment [MH2]: Comment 7

Maximum LBNP tolerance, expressed as CSI (**Figure 1**), improved during the ITD trial (SHAM: 521±306; ITD: 682±324 mmHg min, P=0.003). A significant condition x time interaction was discovered for blood pressure (**Figure 2**, P<0.001). Post-hoc multiple comparisons revealed greater MAP for the ITD experimental trial at T2 (SHAM: 62 ± 9; ITD: 68 ± 10 mmHg, P=0.02) and T3 (SHAM: 50 ± 9; ITD: 66 ± 12 mmHg, P<0.0001). Likewise, the interaction for HR was also significant (**Figure 3**, P=0.023). Post-hoc testing for HR revealed higher heart rates at T3 in the ITD trial (SHAM: 124 ± 33; ITD: 136 ± 19 bpm, P=0.01).

$ETCO_2$ decreased from T1 to T3 in both SHAM and ITD conditions (SHAM: T1 33 ± 7, T2 31 ± 8, T3 28 ± 9 mmHg; ITD: T1 32 ± 8, T2 29 ± 8, T3 28 ± 9; main effect of time, P=0.02); however, there was no difference in $ETCO_2$ between conditions (P=0.52). No differences in respiration rate (RR) were evident between conditions (P=0.23), but RR tended to decrease over time (SHAM: T1, 15 ± 5, T2, 14 ± 4, T3, 15 ± 7 mmHg; ITD: T1, 15 ± 3, T2, 11 ± 4, T3, 12 ± 5; P=0.08), an observation that appears to have been driven by a decline in RR primarily in the ITD trial. CVC was lower for each of the ITD trials at each of the assessed periods (**Figure 4**, P<0.01)

DISCUSSION

The major new finding of this study is that inspiratory resistance breathing improves tolerance to simulated hemorrhage in individuals with elevated internal temperatures. On average, individuals in this study had a ~31% improvement in CSI, an index for maximal tolerance to a progressive LBNP test. In all but one subject,

cardiovascular decompensation, i.e., hypotension and bradycardia, associated with progressive central hypovolemia was delayed during a subsequent experimental trial utilizing the ITD device.

The data from this study agree with findings from previous studies demonstrating the beneficial use of an ITD device during hypotensive conditions, including hemorrhagic shock (Convertino, Ratliff, et al., 2005; Convertino et al., 2007; Victor A. Convertino, Cooke, & Lurie, 2005; Marino et al., 2004; Melby, Lu, Sakaguchi, Zook, & Benditt, 2007; Rickards et al., 2007). Notably, these prior investigations were conducted in normothermic humans. Previously, the effects of an ITD on hypotension during hyperthermia have only been evaluated in swine (Voelckel et al., 2008). In that study, it was revealed that in swine experiencing hypotension caused by heat stroke, blood pressure was immediately increased with the utilization of an ITD device. The current study extends this finding and demonstrates that in hyperthermic humans facing a hemorrhagic insult (simulated via LBNP), the use of an ITD device preserves arterial blood pressure, thereby delaying the precipitous drop in blood pressure that attend presyncope. Thus, the data from this study provides experimental evidence extending the improvements of blood pressure control with inspiratory impedance in hyperthermic conditions to humans.

The ITD counteracts progressive central hypovolemia by decreasing intrathoracic pressure. This leads to greater venous return, stroke volume, cardiac output, and ultimately higher arterial blood pressure (V. A. Convertino et al., 2004, 2007). In hyperthermic conditions, thermoregulatory demands for heat dissipation reduces central blood volume due to profound cutaneous vasodilation (C. G. Crandall et al., 2008, 2012;

C. G. Crandall & Gonzalez-Alonso, 2010; L. B. Rowell, Brengelmann, & Murray, 1969; L. B. Rowell, 1986). The present study attempted to investigate the interplay of this physiological response by measuring cutaneous vascular conductance – an index of cutaneous vasodilation. The data reveal differences in cutaneous vascular conductance between the sham and ITD trials. However, these differences need to be interpreted with caution as the analysis reveals differences in cutaneous vascular conductance two stages (T1) before test termination in the SHAM trial. On the account that the ITD device was not engaged until one stage (T2) before test termination of the SHAM trial, these differences cannot be fully explained by the use of the ITD. Future studies are needed to clarify the previously mentioned cutaneous vasodilatory responses.

The present study represents an exploratory, proof of concept study on the feasibility of improving tolerance to a hemorrhagic challenge during heat stress with inspiratory resistance breathing. Lacking in this study are mechanistic measurements that may give insight into how inspiratory impedance prolongs tolerance to simulated hemorrhage while heat stressed. Rickards et al. provided experimental evidence that the ITD induced larger oscillations in cerebral blood flow velocity that may explain the improvements in LBNP tolerance time (Rickards et al., 2007). In the present study, indices of cerebral blood flow were not measured. However, at the frequency of respiration, greater oscillations in spectral power of blood pressure (SHAM: 0.04 ± 0.03; ITD: 0.26 ± 0.17 $mmHg^2$; P<0.01) and laser Doppler flux (SHAM: 0.25 ± 0.35; ITD: 0.67 ± 0.66 arbitrary $units^2$; P=0.02) were uncovered during the ITD trial (AcqKnowledge 4.2.0, power spectral analysis). However, the interpretation of these data should be

made with the understanding that laser-Doppler indices of skin blood flow were obtained from an exposed forearm wherein skin temperature was not clamped. Responses at this site may be different from oscillations in skin blood flow from the ~85% of the body surface under the water perfused suit. Therefore, the physiological responses for majority of the cutaneous vasculature (i.e., that under the water perfused suit) may not represent responses from skin blood flow outside the water perfused suit.

Comment [MH3]: Comment 4

The extent to which these and other mechanisms (e.g., preserved central blood volume, enhanced venous return, improved cardiac filling, and/or maintained cerebral perfusion), are responsible for the ITD-induced improvements in tolerance need to be further investigated. Furthermore, on the account of the expanding body of evidence suggesting cerebral hypoperfusion does not play as pivotal of a role in the tolerance to central hypovolemia (Ainslie, Hoiland, & Bailey, 2016; Jeong, Shibata, Levine, & Zhang, 2012; Kay et al., 2017; Lucas et al., 2017; Lucas, Pearson, Schlader, & Crandall, 2013) as previously thought, future research directed at elucidating the specific physiological processes that improve tolerance to hemorrhage during heat stress is highly warranted.

A conspicuous constraint of this investigation was that the study design prevented the randomization of the order of the experimental trails. In order to ascertain the proper time to engage the ITD device, tolerance testing without the device must first be conducted. An attempt to address this inherent methodological constraint was the blinding of the participants from the order of experiments. Despite this, the lack of randomization may confound the interpretation of the findings in the form of a training effect. However, given that the improvements in tolerance during the ITD trials were nearly ubiquitous, and prior studies have demonstrated reliable reproducibility of

tolerance to LBNP in normothermia and hyperthermia (V. A. Convertino, 2001; V. A. Convertino & Sather, 2000; Howden, Tranfield, Lightfoot, Brown, & Swaine, 2001; Schlader & Crandall, 2014), even in trials separated by at least 72 hours (Lightfoot, Hilton, & Fortney, 1991), the limitations of this study design restriction appears to be minimal.

Concerning the clinical applications of this study, the data demonstrate a scenario in which exploitation of the respiratory pump can ameliorate serious conditions related to systemic hypotension. These findings indicate the feasibility that an ITD device may be effective in improving life support of the hyperthermic soldier who has a hemorrhagic injury. In conditions that lead to hyperthermia (e.g., prolonged physical activity under environmental heat stress), the use of inspiratory impedance may reduce the incidence of permanent injury or death of the injured soldier by prolonging the treatment window for the soldier to receive medical care.

CONCLUSION

In summary, the results of this study extend previous finding by demonstrating that inspiratory resistance breathing improves tolerance to a simulated hemorrhage challenge in humans with an elevated internal temperature. In addition, the data suggest that this is accomplished by maintaining arterial blood pressure when using an ITD during heat stress and progressive LBNP, thus delaying the onset of presyncope. Future research is needed to evaluate the physiological mechanism responsible for the improved tolerance.

Competing interests

None declared.

Author contributions

All experiments took place at the Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital, Dallas, TX, USA. R.M.B. and C.G.C. contributed to conception and design of the experiments. M.H., R.M.B., M.S.G., R.A.I.L., M.N.C., G.M., V.A.C., and C.G.C. contributed to data acquisition, analysis and/or interpretation of data and experimental results. M.H., R.M.B., M.S.G., R.A.I.L., M.N.C., G.M., V.A.C., and C.G.C. contributed to drafting the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1. Cardiovascular and temperature responses during passive whole-body heating trial.

	NT Baseline		HT (pre-LBNP)	
	SHAM	**ITD**	**SHAM**	**ITD**
Heart Rate (bpm)	64 ± 7	66 ± 12	98 ± 10	98 ± 7
MAP (mmHg)	91 ± 7	85 ± 13	93 ± 9	87 ± 8
Respiratory Rate (breaths/min)	18 ± 2	17 ± 2	17 ± 3	17 ± 4
T_{core} (°C)	37.0 ± 0.2	36.8 ± 0.2	38.3 ± 0.2	38.1 ± 0.3

NT: normothermic; HT: hyperthermic; MAP: mean arterial pressure; LBNP: lower body negative pressure; ITD: impedance threshed device; T_{core}: internal temperature. Comparison of all variables for SHAM vs. ITD revealed $p>0.05$.

Figure 1.

Group averaged (±SD) and individual data showing cumulative stress index as a quantitative measure of maximal tolerance to a graded lower-body negative pressure test for the SHAM and impedance threshold device (ITD) experimental trials.

Figure 2.

Group averaged (±SD) data showing mean arterial pressure at four distinct time points during LBNP. T4, presyncope for the ITD trial. T3, presyncope for the SHAM trial and the corresponding identical time point for the ITD trail; T2, one full LBNP stage prior to T3 – activation of the impedance threshold device (ITD); and T1, two full LBNP stages prior to T3. *Significantly different than SHAM trial ($P<0.05$).

Figure 3.

Group averaged (±SD) data showing HR at four distinct time points during LBNP. T4, presyncope for the ITD trial. T3, presyncope for the SHAM trial and the corresponding identical time point for the ITD trail; T2, one full LBNP stage prior to T3 – activation of the impedance threshold device (ITD); and T1, two full LBNP stages prior to T3. *Significantly different than SHAM trial ($P<0.05$).

Figure 4.

Group averaged (±SD) data showing cutaneous vascular conduction as percentages relative to the value just prior to initiation of LBNP at four distinct time points during

LBNP. T4, presyncope for the ITD trial. T3, presyncope for the SHAM trial and the corresponding identical time point for the ITD trail; T2, one full LBNP stage prior to T3 – activation of the impedance threshold device (ITD); and T1, two full LBNP stages prior to T3. *Significantly different than SHAM trial ($P<0.05$).

Figure 1.

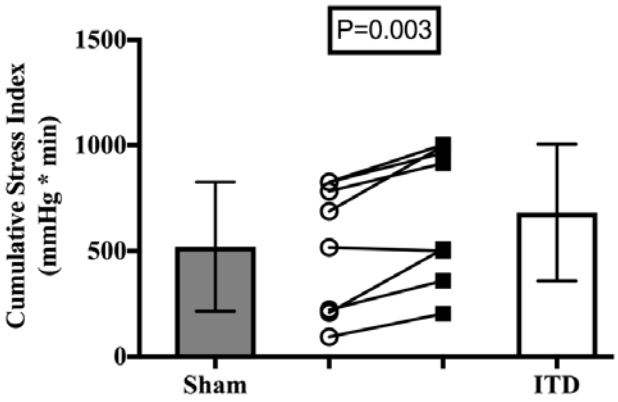

P=0.003
1500
1000
500
0
Cumulative Stress Index
(mmHg * min)
Sham
ITD

Figure 2.

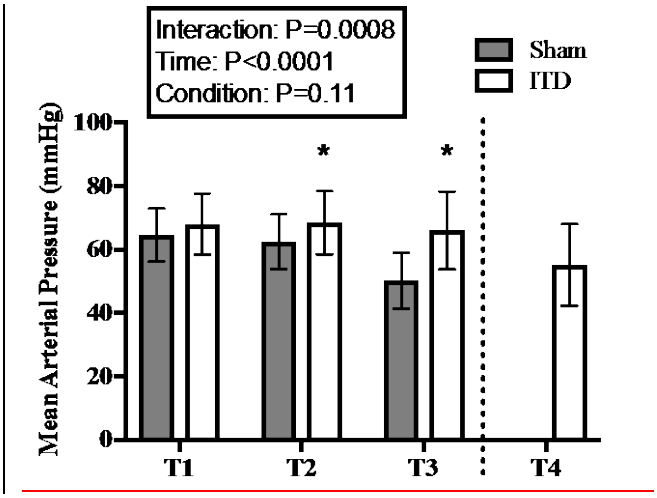

Interaction: P=0.0008
Time: P<0.0001
Condition: P=0.11
Sham
ITD
Mean Arterial Pressure (mmHg)
100
80
60
40
20
0
*
*
T1
T2
T3
T4

Figure 3.

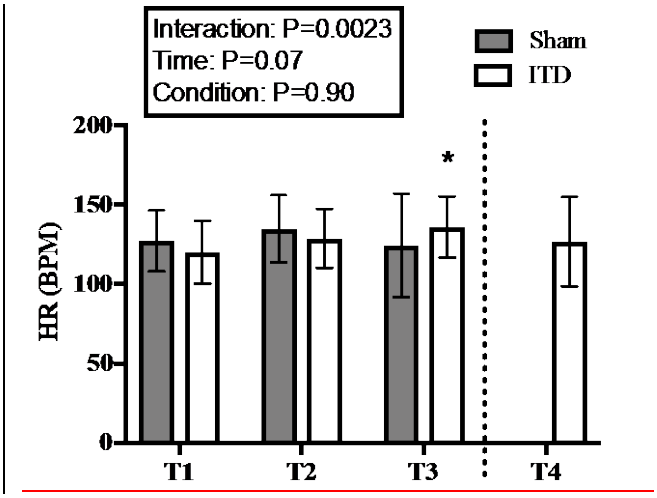
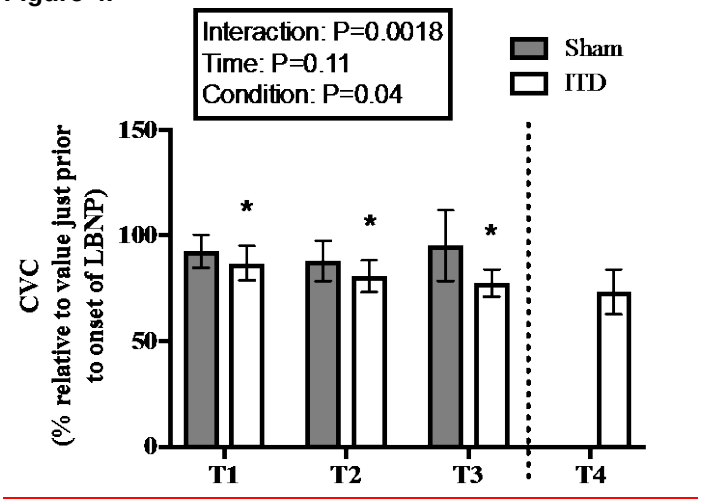

Interaction: P=0.0023
Time: P=0.07
Condition: P=0.90
Sham
ITD
HR (BPM)
200
150
100
50
0
*
T1
T2
T3
T4

Figure 4.


Interaction: P=0.0018
Time: P=0.11
Condition: P=0.04
Sham
ITD
CVC
(% relative to value just prior to onset of LBNP)
150
100
50
0
*
*
*
T1
T2
T3
T4

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